RESEARCH ARTICLE

Obese Gut Microbiome with High Cholesterol Diet Accelerates Obesity in Zebrafish, *Danio rerio* (Hamilton 1822)

C. Justin David*, G. Adaikalaraj
Department of Rural Development Science
Arul Anandar College, Karumathur – 625514
Madurai, Tamilnadu

ABSTRACT: Obesity is a multifactorial disorder induced by a combination of genetic and environmental factors, characterized by chronic accumulation of adipose tissue. Dietary lipids significantly contribute to obesity but regulation of energy homeostasis may be altered by resident gut microbiome. Altering or transplanting gut microbiome may lead to harmful or beneficial effects in humans. Zebrafish has become an excellent vertebrate model for studying dietary lipid absorption and metabolic disorders owing to genetic homology (>80%) and organ similarity to humans, and remain amenable to several genetic manipulation techniques. Our objectives for the present investigation are to study the effects of dietary cholesterol supplemented diet for 8 weeks and assessing the role of gut microbes transplantation from obese zebrafish in inducing obesity. Body Mass Index (BMI) values of obese zebrafish was significantly ($P>0.001$) higher than that of the control. Consumption of fat-rich diet led to increased accumulation and size of lipid droplets in enterocytes and affecting hepatocytes thereby inducing non-alcoholic hepatic steatosis. Interestingly, transfer of gut microbiome from obese to control zebrafish by faecal transplantation through rearing water combined with cholesterol supplementation accelerated adipocyte accumulation and obesity within 5/6 weeks. Role of dietary lipids and gut microbiome in obesity are discussed.

KEYWORDS: High Cholesterol Diet, Gut microbiome, Microbial transplantation, Zebrafish, Obesity.

https://doi.org/10.29294/IJASE.9.1.2022.2599-2606

©2022 Mahendrapublications.com, All rights reserved

INTRODUCTION

Obesity is an epidemic disease ravaging human population in recent years manifesting in clinical complications including Type II diabetes mellitus (T2DM) and several cardiovascular disorders [1]. Consumption of westernised diets rich in fat and sedentary life style are the major contributing factors to obesity in humans [2]. Indian sub-continent is now reeling with many metabolic diseases and has become one of the capitals for obesity and cardiovascular diseases. With increasing industrialization and urbanization, Indian population enjoys more transportation facilities that reduced physical activity and access to fat rich junk diet augmented by sedentary habits leads to exogenous obesity [3]. In addition, life style changes like shift working and couch surfing has significantly increased obesity in young school going children and adolescent population; trend is significantly higher in Trivandrum, Kerala [4]. Obesity is a multifactorial disorder with genetic predisposition resulting in chronic accumulation of fat requiring continuous differentiation of new adipose tissues. In humans, excessive food intake than energy expenditure and reduced physical activity has more effect on regulation of body weight than physiology. Leptin produced in adipocytes regulate lipid metabolism by sending feedback signals to the brain for regulating body weight in humans. Reduction in adipocyte accumulation corresponds to decreased leptin levels, which in turn leads to excess food intake and weight gain [5].

Recent studies have shown that dietary lipid absorption in humans is regulated by the host's gut microbiome. Bacterial populations begin to colonise human gut from birth and results in diverse microbiota composition inhabiting the host until death [6]. Human gut colonized by an enormous number (~10^{14}) of microbes from birth that evolved in parallel and well known to regulate host digestive physiology and energy homeostasis [7]. Several bacterial populations in our gut are well known for their ability to actively ferment nutrients and excrete valuable

---

**Corresponding Author: justindavid.kufos@gmail.com*

Received: 15.062022 **Accepted:** 15.07.2022 **Published on**: 01.08.2022

metabolic by products into the immediate environment and thereby contribute beneficially to human metabolism [8]. Human gut is predominantly colonised by bacterial population from phylum *Firmicutes*, *Bacteroidetes, Actinobacteria* and Proteobacteria [8]. More than 1500 bacterial species are known to inhabit human digestive tracts but only about ~160 species are considered to constitute the core microbiome in humans [9]. Adult gut microbiota responds to dietary changes and in particular, class *Erysipelotrichi* of the phylum *Firmicutes* increases dramatically in the gut within 24 hr of exposure to fat rich diet [10]. Dietary carbohydrates that are not digested in the upper part of the digestive tract may also influence the gut microbial composition [11]. Recently, gut meta-genomics research, where microbial coding sequences of millions of bacterial population are extensively studied, has gained importance owing to the influence of diet on gut microbiome and similarity of microbiome composition between many vertebrate species [12]. Research in gut meta-genomics has shown that many metabolic diseases of the 21st century such as Inflammatory Bowel Syndrome (IBS) is now recognised as the outcome of altered gut microbial content [11-13].

Very recently, researchers have stumbled upon the relationship between gut microbiome composition and obesity in humans. Among several interesting investigations conducted on the composition of gut microbiota and their role in nutrient absorption and metabolism, few have concentrated on the increasing effects of high-fat diet induced microbiome population [6-8]. The study by Ley et al., [14] was one among the few studies that conferred Phylum *Firmicutes* as regulators of energy homeostasis and obesity in humans. The authors identified that Bacterial Phyla *Firmicutes* increased in abundance in obese mice and humans while *Bacteriodetes* population reduced drastically [14-16]. Several studies followed suit and clearly indicated that obesity might indeed be due to altered host gut microbial composition. Interestingly, dietary restriction leading to weight loss of obese individuals is the outcome of altered bacterial composition which results in decreased energy (lipids) uptake and metabolism [17].

Zebrafish has gained importance as an excellent genetic model for studying human metabolic diseases owing to many of its attractive features such as external fertilization, transparent embryos and larvae, short developmental duration (24hr), availability of fully functional organs in <5 days post hatching, organ and genomic similarity to humans and highly conserved physiological mechanisms [18]. Ability of zebrafish embryos to withstand a number of genetic manipulation methodologies has made them an attractive model for biomedical and pharmaceutical research [19-20]. Further, mechanism of dietary lipid absorption and fat metabolism are conserved in zebrafish as in humans and hence can serve as a vertebrate model for studying metabolic disorders [21-22] and cancer [23].Very few studies are conducted successfully on obesity in zebrafish despite the availability of many human disease homologues [24]. Hence, further studies are required for a better understanding of the role of dietary lipids in altering gut microbiome and on host's health and disease. Hence, our objective was to induce exogenous obesity in zebrafish by dietary supplementation of cholesterol and elucidate the beneficial role of gut microbiota by faecal transplantation. We also sought to identify antibiotic resistant microbial colonies resident in zebrafish intestine and whether transplantation of such microbes to control zebrafish may result in obesity. The outcome of the study may be useful in characterizing novel bacterial strains for development of novel pre/probiotics and in identifying potential therapeutic targets and designing novel therapeutic interventions for treating diet-induced obesity and related metabolic disorders.

MATERIALS AND METHODS

Zebrafish and Maintenance

Zebrafish were purchased from an aquarium dealer (M/S. Aqua Farms, Madurai), who has raised a homogenous clonal population of wild type (WT) zebrafish which were used for the present study. Fish were raised under constant environmental conditions (12:12) Light:Dark (LD) and (28 ± 2°C) and fed pelleted diet along with artemia twice a day. Several protocols followed in maintaining the zebrafish population are mentioned in detail elsewhere [22]. All experimental and control zebrafish populations were raised in separate glass tanks carefully avoiding cross contamination of gut microbes through rearing water. The Arul

Anandar College ethical committee approved animal protocols followed in the present study.

Cholesterol Supplemented Diet

Cholesterol (5 %) (Sigma, USA) was dissolved using diethyl ether and mixed with food pellets; pellets were allowed to air dry in sterile conditions inside an incubator (Labmate, India). Preliminary studies clearly showed that 5% cholesterol supplementation for about 15 -16 weeks were sufficient to induce obesity in zebrafish [6, 16]. Air dried pellets were then refrigerated (-20°C) in a metal container and sealed with a tinfoil for the entire duration of the study to prevent bacterial contamination from environment and for preventing cholesterol degradation [6].

Confirmation of Obesity

Male and female zebrafish were weighed prior to the beginning of the scheduled feeding experiments. Experimental zebrafish were fed cholesterol filled pellets, twice a day. About 1/3 volume of water in tanks was regularly changed every other day. Briefly, for measuring weight and length, fish were anesthetized with Clove oil (0.5%), wiped clean with cotton and weighed in an electronic balance (Shimadzu, Japan) and measured with a metal scale. Body Mass Index (BMI) of each fish was calculated by dividing body weight/square of body length [6].

Gut-microbiome isolation, growth and transfer

Control and experimental obese zebrafish were reared separately in glass tanks; care was taken to prevent cross-contamination of water between tanks. Before obtaining faecal pellets, adult obese zebrafish males and females were allowed to swim for 30 min in sterile water. For obtaining faecal pellets, fish were stripped inside a sterile laminar flow hood by slightly pressing their abdomen to release the faecal pellets that were collected in an Eppendorf tube with sterile broth (1 ml), vortexed for 5 min and serially diluted. The colonies were cultured overnight in broth (5 ml) in an incubator shaker (Remi, India) or plated onto agar plates containing antibiotics Ampicillin (*Amp* 100µg), Streptomycin (*Str* 100µg*)* Tetracycline (*Tet* 80µg), Chloramphenicol (*Chlor* 240µg) and Kanamycin (*Kan* 240µg). The obtained faecal gut microbes were then used for transplantation experiments. Genomic DNA isolation for gut meta-genomics studies from resident gut microbial culture was carried out using a DNA isolation Kit (Qiagen, USA).

Briefly, for microbial transfer, Eppendorf tubes with bacterial culture were spun at 13000 rpm in a refrigerated centrifuge (Eppendorf, USA) and bacterial pellets were diluted in 1 ml of sterile broth by gentle aspiration. The diluted cultures were mixed with sterile water (~1Lr) in a plastic container and experimental zebrafish were allowed to swim for a couple of hours (3-4 hr) and transferred to separate tanks while the control is maintained in separate tanks. Further, a simple methodology was also attempted for faecal transplantation by transferring ~50 ml of rearing water from obese zebrafish tanks to tanks containing experimental zebrafish. This simple bacterial cross contamination methodology can be applied in several farmed economically important edible fish species.

Histological analysis of obese zebrafish liver and intestine

Control and obese zebrafish were sacrificed and visceral organs fixed in Bouins fixative overnight for histological analysis. Liver and intestine were cross sectioned and stained with Haematoxylin for identifying diet-induced Non-Alcoholic Fatty Liver and lipid droplets inside enterocytes of intestine, respectively. Enlarged sections of gut microvillus were counted for the increased presence of oil droplets between control and obese zebrafish [25].

Confirmation of Obesity

In the present study, obesity was confirmed by measuring Body Mass Index (BMI) values in zebrafish as in humans. Obese zebrafish showed significantly increased fat accumulation in the abdomen (Fig. 1-I) which is more pronounced in females than in males. Obese zebrafish showed behavioural symptoms like reduced physical activity and surfacing. Body Mass Index (BMI) values of obese zebrafish males (0.059) and females (0.071) were significantly higher than the control

Histopathology of obese zebrafish enterocytes and hepatocytes

From the present study, we know that zebrafish fed high cholesterol diet are prone to become obese and display related co-morbidities like increased accumulation of lipid droplets in intestinal lumen (Fig. 2 B, C) and in hepatocytes resembling symptoms for non-alcoholic fatty liver (Fig. 2 A) as in humans. Lipid deposits observed in hepatocytes of obese zebrafish liver were higher than the control and hepatocytes remain enlarged after increased consumption of the cholesterol diet.

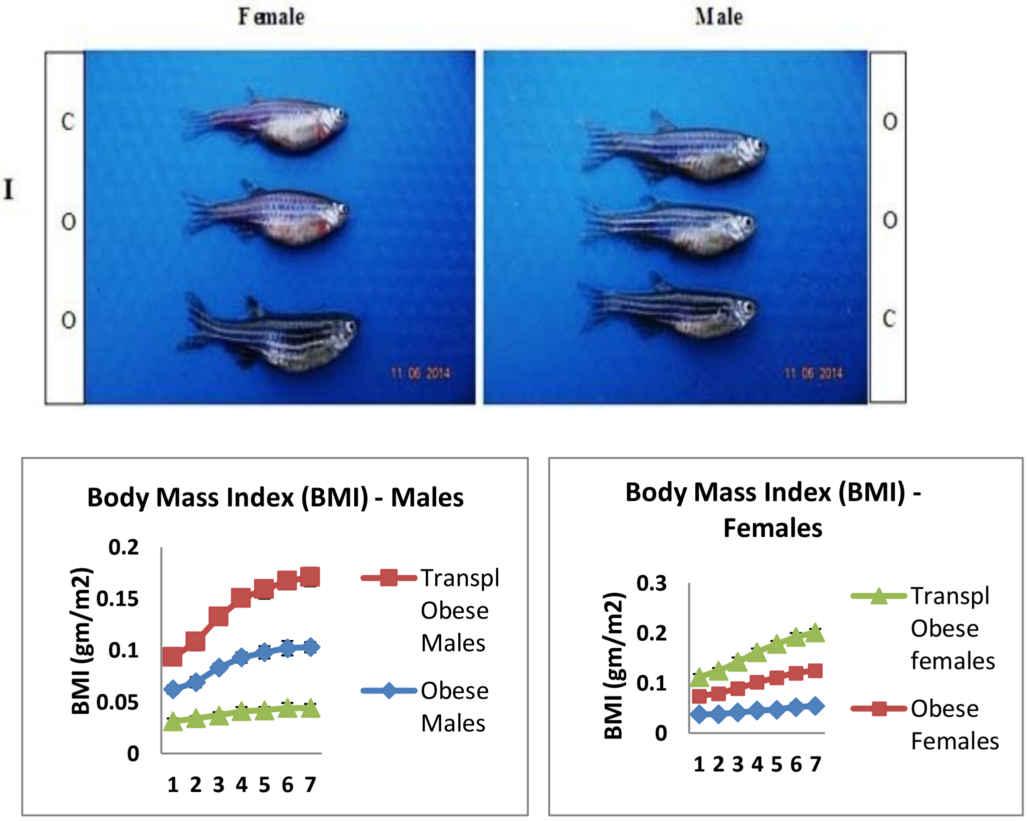

Figure 1 Diet induced Obese zebrafish: I. female (c)- control; (o) - Obese and (B) male (c)- control (o) – Obese II. Graphs showing (1) BMI values of Control, Obese and microbiome transplanted males and (2) Female zebrafish induced by diet supplemented with Cholesterol and Cholesterol diet-supplementation with microbial transplantation from obese zebrafish.

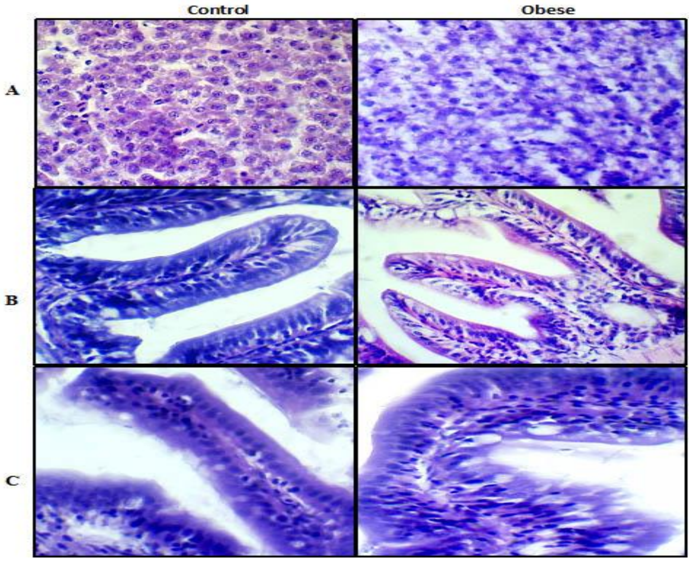

Figure 2 Histo-pathology: Cross sections of (A) liver showing diet-induced non-alcoholic hepatosteatosis in liver of control and obese zebrafish hepatocytes. (B) Accumulation of lipid droplets in the intestinal lumen of control and obese zebrafish. (C) Enlarged view of intestinal villi showing accumulation of lipid droplets in control and obese zebrafish intestine.

Behavioural analyses were also carried out to determine physical activity pattern of obese and control zebrafish population. Obese zebrafish were reluctant to move faster and escape when threatened with hand held fishing net while the control zebrafish were able to move faster and escape. When obese and control zebrafish were subjected to swimming exercise using a magnetic stirrer inside a beaker (1 litre), obese zebrafish were not able to swim against the current and even died while the controls were able to maintain a steady swimming rhythm against the current (data not shown).

When gut microbes were plated onto agar plates containing antibiotics such as Ampicillin (*Amp*), Streptomycin (*Str)* Tetracycline (*Tet*), Chloramphenicol (*Chlor*) and Kanamycin (*Kan*), some antibiotic resistant colonies were found to grow in control plates rather than in the obese (Fig. 3). From the present study, it may be concluded that in zebrafish, gut microbiome may be transferred from both parents to the offspring through rearing water, which contains faecal pellets of both parents thus imparting immunity to infections. .

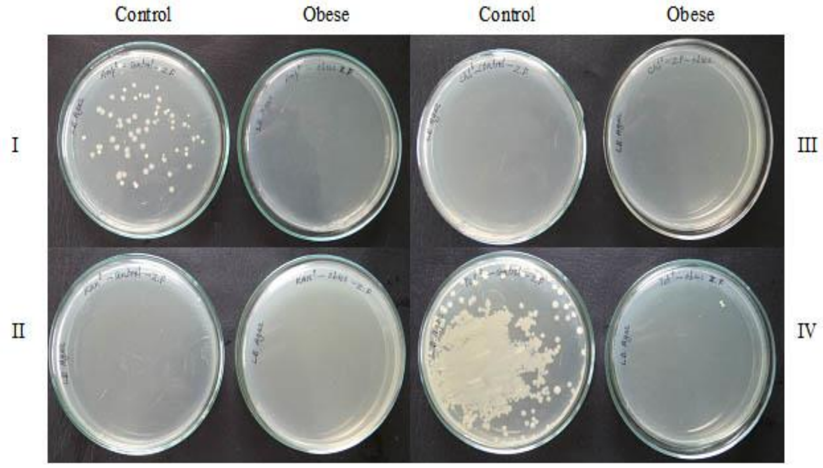

Figure 3 Plates showing growth of microbial colonies isolated from faecal pellets of zebrafish. Bacterial colony in plates isolated from control and Obese intestine: with antibiotics (I) Ampicillin (II) Kanamycin (III) Chloramphenicol and (IV) Tetracycline.

Hence, zebrafish can serve as a valuable model for gut meta-genomics research if fertilization and embryonic development are completed *in vitro* in a sterile environment for generating gnotobiotic strains [16, 26]. Our present study also confirms the significant role of gut microbiome in accelerating obesity induction in zebrafish. We conclude that microbes transferred from faecal pellets through rearing water to young larvae can alter colonisation of gut microbiome in adult zebrafish and remain a good source for transplantation and accelerating obesity along with cholesterol diet which can be applied to many farmed economically important edible fishes. Despite our best efforts, several attempts to generate gnotobiotic zebrafish failed due to unavailability of sterile space that required separate laminar flow hood for maintaining such gnotobiotic population in isolation [16]. However, provided such necessities are available, generating gnotobiotic strains of zebrafish may be feasible and useful for identification and characterization of microbial population for production of prebiotics and probiotics beneficial to humans [9, 27].

DISCUSSION

Obesity, characterized by chronic accumulation of adipose tissues in humans is a non-communicable, multifactorial metabolic disorder ravaging human population in both developed and developing countries [2]. WHO reports that ~ 1/3 of the world population are now obese and India which was earlier known to have more lean population are now leading the chart for metabolic disorders like obesity, Type II diabetes and cardiovascular diseases [4]. Many developing countries with ever-increasing job opportunities that demand work shifts and sedentary life style have more obese population with increased body mass index due to consumption of fat-rich diets and altered internal biological clock [6]. In the present study, obese zebrafish exhibited both physiological pathways and behavioural pattern similar to humans. The results clearly indicate that dietary lipids significantly contribute to obesity induction and alter gut microbiome in zebrafish. Hence, the present study can be considered among the few investigations conducted using a zebrafish model for better understanding of the effects of fat-rich westernised diets upon gut microbiome composition, lipid metabolism and obesity induction [16, 26-27].

Study conducted by Sahota et al. [28] among school going children has clearly indicated an alarming rise in obesity among young juvenile and paediatric populations. With the advent of electronic appliances that removed physical activity among homemakers has led to a dramatic increase in obesity especially among young women culminating in several clinical manifestations during pregnancy and delivery [29-30]. While calorie restriction and physical exercise in young adolescents play a positive role in reducing adipocyte deposition many have pointed out that several genetic factors contribute to obesity especially, genetic polymorphism in genes controlling appetite, lipid metabolism and factors such as adiponectin are well known predisposing factors for obesity [4,6, 22]. Earlier studies have shown that decrease in leptin levels led to increased food intake in young adolescents [5]. For instance, hormone leptin, which is known to regulate body weight, metabolism and reproductive function in humans might play the same role in zebrafish as our preliminary studies support the same [6]. As obesity requires relentless differentiation of adipocytes, which is under the control of Peroxisome Proliferator Receptor Gamma (PPARγ) [24], it is essential to understand the gene expression profile in obese models hence transcriptome profiling to identify genes up or down regulated in obese is underway.

Mammalian embryos undergo internal fertilization and embryonic development within placenta but receive microbiome from the mother at the time of birth hence, gut of new born babies will have microbial colonies resembling maternal gut microbiome [8]. In contrast, zebrafish embryos undergo external fertilization and embryonic development but exposed to many environmental pathogens and pollutants at a very early stage beginning from fertilization [9,16,27] .Colonisation of gut microbes in zebrafish occurs at a very early stage of development (>3 days post hatching, dph). The present study clearly confirms that increased consumption of diet supplemented with cholesterol lead to obesity in zebrafish similar to humans and affects gut microbial composition in adults [26]. Nevertheless, it remains unclear whether gut microbiome composition is determined by the offspring's genome or purely through transfer from the mother after birth and whether they are susceptible to alteration by changes in diet.

Gut microbiota profiling in zebrafish is an arduous task due to inability of many microbes to grow outside gut environment and complications in raising pure strains of contributing microbes *in vitro* for biochemical and genetic analysis [26,27]. Recent studies have suggested that genetic background, sex, diet and age of the host determine the gut microbiome composition which defines host immunity in humans [4]. Rawls et al., [27] created gnotobiotic strains of zebrafish for identifying the role of gut microbes in host health and disease however, gnotobiotic fish failed to survive owing to immunodeficiency against pathogens. Since, microbes that colonise gut have a significant effect on host energy metabolism by altering expression of genes involved in lipid absorption, adiposity and related metabolic complications this field of research merits further study [31, 32]. Identification of novel therapeutic targets in obese and lean animals including zebrafish may lead to anti-obesity therapy in humans [32]. Further, identification of novel gut bacterial strains and their metabolic by products using zebrafish may contribute to human health and in developing novel pre and pro-biotic strains beneficial to humans.

CONCLUSION

Our present study demonstrates the feasibility of creating a diet-induced obese zebrafish model for understanding the molecular mechanisms orchestrating human obesity. The study clearly indicates the imminent role of gut microbiome in accelerating the digestive physiology especially of lipids thereby increasing the deposition of lipid as droplets in the enterocytes and hepatocytes phenocopying non-alcoholic heapatosteatosis. However further research is required to understand the beneficial role of gut microbes in infection and immunity which may also pave way for isolation and characterization of novel microbiome population for development ofprobiotics for human benefit.

Acknowledgements

The author (Dr. C.J. David) thanks DST-SERB, New Delhi for the financial support and gratefully acknowledge support of Arul Anandhar College, Jesuit Management. The author would like to thank Prof. Dr. Arockiaraj, HoD, Department of Rural Development Science and the Arul Anandar College for providing necessary infrastructure and support.

REFERENCES

[1] Stein, C.J.,G.A. Colditz, 2004. The epidemic of obesity. Journal of Clinical Endocrinology and Metabolism, 89, 2522-2525.

[2] Ogunbode, A.M., Ladipo, M.M.A, Ajayi, I.O., Fatiregun. A.A. 2011. Obesity: An emerging disease. Nigerian Journal of Clinical Practice, 4, 390-394.

[3] Anate, M., Olatinwo, A.W., Mesina, A.P. 1998. Obesity an overview. West African Journal of Medicine, 17, 248-254.

[4] Ambika, G.U., K. Sanjay and M.K. Garg.2012. Preventing Obesity in India: weighing the options, 16(1) 4-6.

[5] Oyekan, A.O.2005.Genetics of food intake, body weight and obesity-role of leptin, Nigerian Medical Practitioner, 47, 108-112.

[6] Justin David, C., Rajapriya, R., Veena, V., Kumaresan, G. 2016. High cholesterol diet induces obesity in zebrafish. Intl. J.Adv.Sci. Eng., 2 (4) 188-192.

[7] Reinhardt, C., Reigstad C.S, Backhed. F. 2009.Intestinal microbiota during infancy and its implications for obesity. Journal of Pediatric Gastroenterology and Nutrition, 48, 249-256.

[8] Delzenne, N.M., A.M. Neyrinck., F. Backhed and P.D. Cani.2011. Targeting gut microbiota in obesity: effects of prebiotics and probiotics. Nature Reviews Endocrinology 7, 639-646.

[9] Manichanh, C., J. Reeder., P. Gibert., E. Varela., M. Liopis., M. Antolin., R. Guigo., R. Knightand F. Guarner.2010. Reshaping the gut microbiome with bacterial transplantation and antibiotic intake. Genome Research, 20, 1411-1419.

[10] Junjie Qin, Ruiqiang Li, Jeroen Raes, Manimozhiyan Arumugam, Kristoffer Solvsten Burgdorf, Chaysavanh Manichanh, Trine Nielsen, Nicolas Pons, Florence Levenez, Takuji Yamada, Daniel R Mende, Junhua Li, Junming Xu, Shaochuan Li, Dongfang Li, Jianjun Cao, Bo Wang, Huiqing Liang, Huisong Zheng, Yinlong Xie, Julien Tap, Patricia Lepage, Marcelo Bertalan, Jean-Michel Batto, Torben Hansen, Denis Le Paslier, Allan Linneberg, H Bjørn Nielsen, Eric Pelletier, Pierre Renault, Thomas Sicheritz-Ponten, Keith Turner, Hongmei Zhu, Chang Yu, Shengting Li, Min Jian, Yan Zhou, Yingrui Li, Xiuqing Zhang, Songgang Li, Nan Qin, Huanming Yang, Jian Wang, Søren Brunak, Joel Doré, Francisco Guarner, Karsten Kristiansen, Oluf Pedersen, Julian Parkhill, Jean Weissenbach, 2010. Human gut microbial gene catalogue established metagenomic sequencing. Nature, 464, 59-65.

[11] Turnbaugh, P.J., Backhed, F., Fulton, L. Gordon. J.I. 2008. Diet induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell Host and Microbe, 3, 213-223.

[12] Hildebrandt, M.A, Hoffman, C., Sherrill-Mix., S.A., Keilbaugh, S.A., Hamady, M. Chen, Y.Y., Knight, R., Ahima, R.S., Bushman F., Wu. G.D. 2009. High fat determines the composition of the murine gut microbiome independently of obesity. Gastroenterology, 137, 1716-1724.

[13] Ott, S.J., Musfeldt, M., Wenderoth, D.F., Hampe, J., Brant, O., Folsch, U.R., Timmis K.N., Schreiber, S. 2004. Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease, Gut, 53, 685-693.

[14] Ley, R.E., Backhed, F., Turnbaugh, P., Lozupone, C.A., Knight R.D., Gordon. J.I. 2009. Obesity alters gut microbial ecology. Journal of Pediatric Gastroenterology and Nutrition. 102, 11070-11075.

[15] Turnbaugh, P.J., Ley, R.E., Mahowald, M.A., Mangrini, V., Mardis, E.R., Gordon, J.I. 2006. An obesity associated gut

microbiome with increased capacity for energy harvest. Nature, 444**,** 1027-1031.

[16] Justin D Clifton., Vimal, P., Veena, V.V., Rajapriya, R., Pandian T.J.2016. Preliminary investigations on gut microbes for developing gnotobiotic zebrafish. Int. J. Adv. Sci. Eng. 2(3) 138-140.

[17] Furet, J.P., Kong, L.C., Tap, J. Poitou., C. Basdevant., A. Bouillot, J.L., Mariat, D., Corthier, G., Dore, J., Henegar, C., Rizkalla, S., Clement, K. 2010. Differential adaptation of human gut microbiota to bariatric surgery-induced weight-loss: links with metabolic and low grade inflammation markers. Diabetes, 59**,** 3049-3057.

[18] Santoriello, C., Zon. L.I. 2012. Hooked! Modelling human disease in zebrafish. Journal of Clinical Investigation, 122 (7) 2337-2343

[19] Langheinrich, U. 2003. Zebrafish: a new model on the pharmaceutical catwalk. Bioessays. 25(9) 904-912.

[20] Taylor, A.M., Zon, L.I. 2011.Modelling diamond black fan anaemia in the zebrafish. Seminars in Hematology. 48(2) 81-88.

[21] Farber, S.A., Pack, M., Ho, S.Y., Johnson, I.D., Wagner, D.S., Dosch, R., Mullins, M.C., Hendrickson, H.S., Hendrickson, E.K., Halpern, M.E. 2001. Genetic analysis of digestive physiology using fluorescent Phospholipid reporters. Science. 292, 1385-1388.

[22] Clifton, J.D., Lucumi, E., Myers, M.C., Napper, A., Hama, K., Farber, S.A,. Smith, A.B. Huryn, D.M., Diamond S.L., Pack. M. 2010.Identification of novel inhibitors of dietary lipid absorption using zebrafish. Public Library of Science. 5 (8**)** e12386.

[23] Langenau, D.M., Traver, D., Ferrando, A.A., Kutok, J.L., Aster, J.C., Kanki, J.P., Lin, S., Prochownik, E., Trede, N.S., Zon L.I., Look, A.T. 2003. Myc-induced T Cellleukemia in transgenic zebrafish. Science, 299 (5608**)** 887-890.

[24] Vidal-Puig, A.J., Considine, R.V., Jimenez-Linan, M., Werman, A., Pories, W.J,. Caro J.F., Flier. J.S. 1997. Peroxisome Proliferator-activated receptor gene expression in human tissues: Effects of obesity, weight loss and regulation by insulin and glucocorticoids. Journal of Clinical Investigation, 99, 2416-2422.

[25] Clifton J. David., Pandian, T.J. 2016. High Cholesterol Diet (HCD) induces non-alcoholic fatty liver disease (NAFLD) in Zebrafish, Eur. J. Biomed.Pharm. Sci. 3(6) 431-435.

[26] Semova, I., Carten, J.D., Stombaugh, J,. Mackey, L.C., Knight, R., Farber S.A., Rawls. J.F. 2012. Microbiota regulates intestinal absorption and metabolism of fatty acids in the zebrafish. Cell Host Microbe, 12:277-288.

[27] Rawls, J.F., Samuel, B.S., Gordon. J.I. 2004.Gnotobioticzebrafish reveal evolutionarily conserved responses to the gut microbiota. Proceedings of the National Academy of Science, 101, 4596-4601.

[28] Sahota, P., Rudolf, M.C., Dixey, R., Hill, A.J., Barth, J.H., Cade. J. 2001. Evaluation of implementation and effect of primary school based intervention to reduce risk factors for obesity. British Medical Journal, 323**,**1027-1032.

[29] Rakel, E.R. 2002.Nutrition and Family Medicine. In Textbook of Family Practice. (Ed. Rakel R.E.) 6th Ed. Philadelphia: Saunders: 1107-1058.

[30] Singh, R.B., Pella. D. 2007. Prevalence of obesity, physical inactivity and under nutrition, a triple burden of diseases during transition in a developing economy. The five city study group. Acta Cardiologica, 62, 119-127.

[31] Ley, R.E., Turnbaugh, P.J., Klein, S,. Gordon, J.I. 2006. Microbial Ecology: human gut microbe associated with obesity. Nature 444, 1022-1023.

[32] Turnbaugh, P.J., Hamady, M., Yatsunenko, T., Cantarel, B.L., Duncan, A., Ley, R.E., Sogin, M.L., Jones, W.J., Roe, B.A., Affourtit, J.P., Egholm, M., Henrissat, B., Heath, A.C., Knight, R., Gordon. J.I. 2009.A core gutmicrobiome in obese and lean twins. Nature, 457, 480-484.

All © 2022 are reserved by International Journal of Advanced Science and Engineering. This Journal is licensed under a Creative Commons Attribution-Non Commercial-ShareAlike 3.0 Unported License.